

We claim:

1. An automated method for analyzing neurite outgrowth comprising  
-providing an array of locations comprising cells, wherein the cells possess at least  
a first luminescently labeled reporter molecule that reports on cell location, and at least a  
5 second luminescently labeled reporter molecule that reports on neurite outgrowth, and  
wherein the cells comprise neurons;

-imaging or scanning multiple cells in each of the locations containing multiple  
cells to obtain luminescent signals from the first and second luminescently-labeled reporter  
molecule;

10 -converting the luminescent signals into digital data; and

-utilizing the digital data to automatically make measurements, wherein the  
measurements are used to automatically calculate changes in the distribution, environment  
or activity of the first and second luminescently labeled reporter molecules on or within the  
cells, wherein the calculated changes provide a measure of neurite outgrowth from the  
15 neurons.

2. The method of claim 1 further comprising contacting the neurons with a test  
compound, and wherein the calculated changes indicate whether the test compound has  
modified neurite outgrowth in the neurons.

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3. The method of claim 2, further comprising contacting the neurons with a  
neurotoxin either before, after, or simultaneously with the test compound.

4. The method of claim 1 wherein the first luminescently labeled reporter molecule  
25 comprises a DNA binding compound.

5. The method of claim 1 wherein the second luminescently labeled reporter molecule  
comprises a compound that selectively detects a cell component selected from the group  
consisting of cytoplasm, membrane, neuron-specific cell component, and cellular proteins.

6. The method of claim 1, further comprising contacting the cells with a control compound known to stimulate neurite outgrowth, and utilizing the calculated changes to determine whether the test stimulus inhibited the control compound from inducing neurite outgrowth in the neurons.
7. The method of claim 1 wherein the calculated changes are used to identify conditions that are toxic to neurons and affect neurite morphology.
8. The method of claim 1 wherein the second luminescently labeled reporter molecule is neuron-specific.
9. The method of claim 1 wherein the array of locations containing cells include cells other than neurons, and wherein the neurons possess a neuron-specific luminescent reporter molecule, and wherein the neuron-specific luminescent reporter molecule is spectrally distinguishable from the first and second luminescently labeled reporter molecule.
10. The method of claim 8 or 9 wherein the neuron-specific luminescent reporter molecule comprises a molecule selected from the group consisting of neurofilament proteins,  $\beta$ III-tubulin, ciliary neurotrophic factor, and antibodies specific for neurofilament proteins,  $\beta$ III-tubulin, ciliary neurotrophic factor.
11. The method of claim 1, wherein the imaging or scanning comprises the steps of:
- acquiring a nuclear image and a neurite image;
  - identifying cell bodies; and
  - identifying neurites extending from each cell body.
12. The method of claim 11 wherein identifying cell bodies comprises the steps of:

a. generating a kernel image from the nuclear image;  
b. performing conditional dilations of the kernel image to identify the cell body.

5 13. The method of claim 12; wherein identifying neurites extending from cell bodies comprises the steps of:

a. generating a reservoir image from the neurite image; and  
b. identifying positive pixels in the reservoir image that are not present in the cell bodies, wherein such positive pixels belong to neurites extending from cell bodies.

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14. The method of claim 13, further comprising

a. performing one conditional dilation of the kernel image to acquire a dilation image;

b. determining a set of nodes from the dilation image;

15 c. linking together connected nodes; and

d. repeating steps (a)-(c) until an entire neurite length has been traced.

15. The method of any of claim 1-9, 11-14, wherein the measurements include one or more of the following:

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a. Number of cells;

b. Number of neurons;

c. Total neurite length from all cells;

d. Total number of neurite branches from all cells;

e. Number of neurites per cell;

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f. Number of neurites per positive neuron

g. Neurite length from each cell;

h. Neurite length per positive neuron

i. Neurite length per neurite

j. Number of cells that are positive for neurite outgrowth

- k. Percentage of cells positive for neurite outgrowth;
- l. cell body area;
- m. number of branches per neuron;
- n. number of branches per neurite; or
- 5 o. Degree of neurite outgrowth from a neuron or neuronal cell cluster.

16. The method of claim 15, wherein the calculated changes include one or more of the following:

- a. Changes in the number of cells;
- 10 b. Changes in the number of neurons;
- c. Changes in the total neurite length from all cells;
- d. Changes in the total number of neurite branches from all cells;
- e. Changes in the number of neurites per cell;
- f. Changes in the number of neurites per positive neuron
- 15 g. Changes in the neurite length from each cell;
- h. Changes in the neurite length per positive neuron
- i. Changes in the neurite length per neurite
- j. Changes in the number of cells that are positive for neurite outgrowth
- k. Changes in the percentage of cells positive for neurite outgrowth; or
- 20 l. Changes in cell body area;
- m. Changes in number of branches per neuron;
- n. Changes in number of branches per neurite; or
- o. Changes in the degree of neurite outgrowth from a neuron or neuronal cell cluster.

25 17. The method of claim 1, wherein sub-regions of the array of locations containing cells are sampled multiple times at intervals to provide kinetic measurement of changes in the distribution, environment or activity of the luminescent reporter molecules on or within the cells

18. A computer readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute the method of claim 1 wherein the cell screening system comprises an optical system with a stage adapted for holding a plate containing cells, a means for moving the stage or the optical system, a digital camera,  
5 a means for directing light emitted from the cells to the digital camera, and a computer means for receiving and processing the digital data from the digital camera.

19. A kit for analyzing neurite outgrowth comprising:

(a) at least one neuron-specific luminescent reporter molecule;

10 (b) a nucleus-specific luminescent reporter molecule; and

(c) instructions for using the neuron-specific luminescent reporter molecule and the nucleus-specific luminescent reporter molecule to analyze neurite outgrowth.

20. The kit of claim 19 wherein the neuron-specific luminescent reporter molecule  
15 comprises a molecule selected from the group consisting of neurofilament proteins,  $\beta$ III-tubulin, ciliary neurotrophic factor, and antibodies specific for neurofilament proteins,  $\beta$ III-tubulin, ciliary neurotrophic factor.

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